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Outbreak of Gastroenteritis in Military Recruits Associated With Serotype 3 Astrovirus Infection

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A serotype 3 astrovirus was identified in stool samples from an outbreak of acute gastroenteritis that occurred among military recruits in France. Sixteen stools samples and eight pairs of acute- and convalescent-phase serum were collected from affected individuals. Astrovirus was detected in two stool samples by electron microscopy and in four stool samples by reverse transcriptase-polymerase chain reaction (RT-PCR). Seroconversion to the astrovirus present in one stool was detected in seven patients by using solid-phase immune electron microscopy (SPIEM) and dot blot. For three patients, the serological results were consistent with the PCR results, indicating that astrovirus was a cause of gastroenteritis in these young adults. This study describes the characterization of the serotype 3 astrovirus associated with this outbreak. The virus has a buoyant density in cesium chloride of 1.365 gm/ml and contains two proteins immunoprecipitated with rabbit serum. Only the larger protein was recognized by immunoblotting using a convalescent-phase human serum. The protein composition of this virus differs from that reported for serotype 1 astrovirus, indicating heterogeneity in the capsid composition among astrovirus serotypes. ***J Med Virol 51:101–106, 1997.*** © 1997 Wiley-Liss, Inc.*

KEY WORDS: astrovirus; RT-PCR; immune EM

INTRODUCTION

Astroviruses were described first by Madeley and Cosgrove (1975a) as 28 nm particles with a characteristic star-shaped motif when visualized by electron microscopy (EM). Astrovirus strains have been adapted to growth in continuous cell lines with a strict requirement for medium containing trypsin for continuous virus propagation (Lee and Kurtz, 1981; Willcocks et al., 1990). A recent examination of the structure of astrovirus propagated in cell culture reported the diameter as 31 nm with 10 nm spikes (Risco et al., 1995).

The astrovirus genome is a single-strand positive-sense RNA with three open reading frames (ORF), ORF1a, ORF1b, and ORF2 (Jiang et al., 1993; Monroe et al., 1993; Willcocks et al., 1994). ORF1a and ORF1b encode putative protease and polymerase domains, the latter of which is expressed as a fusion protein synthesized via a ribosomal frame-shift mechanism (Lewis and Matsui, 1995; Marczinke et al., 1994). ORF2 encodes the viral capsid protein precursor (Lewis et al., 1994). During astrovirus replication, a 2.7 kb subgenomic RNA is synthesized in infected cells (Monroe et al., 1993, 1991b). This subgenomic RNA, which includes ORF2, probably serves as an mRNA for the expression of the capsid proteins during infection.

Seven serotypes of human astrovirus have been distinguished by using immune EM (IEM) or immunofluorescence (Kurtz and Lee, 1984; Lee and Kurtz, 1994). In the United Kingdom, serotype 1 was reported to be the most prevalent type (Lee and Kurtz, 1994), and we have recently confirmed and extended this finding by using collections from various regions of the world (Noel et al., 1995). This study went on to show that nucleotide sequences in one part of ORF2 define seven genotypes that are completely consistent with the serotypes determined by EIA (Noel et al., 1995).

Few studies have reported on the protein structure of the astrovirus capsid (Herring et al., 1981; Shimizu et al., 1990; Kurtz and Lee, 1987). Different patterns have been observed depending on host species and serotype. Initially, four proteins (32 to 36 kDa) were reported for human astrovirus (Kurtz and Lee, 1987). More recently, three capsid proteins (20 to 33 kDa) were described for human serotypes 1 and 2 (Willcocks et al., 1990; Monroe et al., 1991b), whereas a serotype 5 strain, Marin County agent, is composed of a unique 30 kDa protein (Midthun et al., 1993). In the one report where

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processing of astrovirus capsid proteins was examined, three major capsid proteins were detected in serotype 2, but two of these were shown to have overlapping sequences (Sanchez-Fauquier et al., 1994).

Astrovirus is a cause of diarrhea world wide, with detection rates reported between 5 and 9% of sporadic cases in young children (Lew et al., 1990; Herrmann et al., 1988). Outbreaks of gastroenteritis associated with astrovirus infection have been reported in the elderly (Midthun et al., 1993; Lewis et al., 1989) and in child care centers or schools, where adult staff are often affected (Oishi et al., 1994; Mitchell et al., 1995). Astrovirus has been shown recently to be significantly associated with diarrhea in HIV-infected patients (Grohmann et al., 1993). The role of astrovirus in episodes of gastroenteritis in healthy young adults remains unclear. There was a very low rate of symptomatic infection in two separate trials with human volunteers (Kurtz et al., 1979; Midthun et al., 1993).

In this paper, we report that astrovirus can be a causative agent of gastroenteritis in healthy military recruits. We also report the characterization of a serotype 3 astrovirus strain associated with this outbreak.

MATERIALS AND METHODS

Clinical Specimens

An outbreak of gastroenteritis with predominant symptoms of diarrhea and vomiting occurred in military recruits in Caen, France in 1991. Sixteen stools and 8 pairs of acute- and convalescent-phase serum were collected. Stool samples were cultured for routine enteric bacteria, were examined by negative stain EM, and were tested for group A rotavirus by using the EIA kit (IDEIA, DAKO).

Reverse Transcriptase/Polymerase Chain Reaction

RNA was purified from 50 μl of freon-extracted 10% stool suspension prepared in phosphate-buffered saline (PBS), pH 7.2, by using guanidinium isothiocyanate (Sigma). Purified RNA was resuspended in 20 μl of diethyl pyrocarbonate-treated water and was used for cDNA synthesis. Five microliters of the RNA solution were reverse transcribed by using 10 units of AMV-RT (Promega) with 50 pmoles of oligo$(dT)_{18}$ (Promega) in a final volume of 25 μl for 1 hour at 42°C. The polymerase chain reaction (PCR) was performed by using 5 μl of cDNA with 0.5 units of *Taq* DNA polymerase (Appligene) and 25 pmol of each primer, in a final volume of 50 μl. Enterovirus PCR was done by using the primers described by Zoll et al (Zoll et al., 1992), which give an amplicon of 400 bp from the 5′ untranslated region (UTR). Detection of astroviruses was done by using primers Mon2 and Mon67 (Mitchell et al., 1995), which give an 89 bp amplicon from the 3′ UTR of the astrovirus genome. The products of PCR were analyzed by electrophoresis in 1% (EV) or 4% (astrovirus) neutral agarose gels and were visualized by UV illumination after staining with ethidium bromide.

Cell Culture and Virus Purification

Astroviruses were cultured from stool samples in Caco-2 cells as described by Willcocks et al. (1990). The cell lysates were harvested 3–6 days postinfection. An astrovirus isolated from the stool of patient 7, strain A7, was selected for large-scale growth and purification. Virus was purified from the cell lysate of ten 75 cm^2 flasks. The pooled lysate was extracted with freon, and the supernatant containing the virus was centrifuged for 3 hours at ×120,000*g* (SW28; Beckman). The resulting pellet was resuspended in PBS, pH 7.4, and was extracted with freon and centrifuged on a cesium chloride gradient with an initial density of 1.36 grams/ml in PBS, pH 7.4, for 40 hours at ×16,0000*g* (SW50; Beckman). Fractions of 200 μl were collected, the absorbance at 260 and 280 nm was measured, and the density was calculated from the refractive index. Fractions enriched for astrovirus particles, as determined by EM, were used for dot blot analysis and polyacrylamide gel electrophoresis (PAGE; Laemmli, 1970).

The enterovirus present in stool 12 (E12) was isolated in Caco-2 cell culture. The virus was purified from infected cell lysates as described for astrovirus.

Solid- Phase IEM

The acute- and convalescent-phase sera were diluted 1:50 in PBS, pH 7.4, and incubated with the stool of patient 7. The degree of virus aggregation was estimated by EM.

Dot Blot Protein Hybridization

Twenty microliters of purified astrovirus A7 or enterovirus E12 were spotted in duplicate onto a nitrocellulose membrane (Fig. 2). Acute- and convalescent-phase sera were diluted 1:500 in Tris-buffered saline (TBS), pH 8, with 1% nonfat dry milk and were incubated overnight with the immobilized antigen. A secondary antihuman antibody (Sanofi Pasteur) labeled with peroxidase was incubated with the membranes for 1 hour at room temperature. Peroxidase activity was detected by incubation of the membranes in 100 ml of 0.6 mg/ml chloronaphtol, 75 μl of 30% hydrogen peroxide in TBS, pH 8. After a 5 minute incubation at room temperature, the reaction was stopped by washing in water.

Serotyping and Genotyping

The serotyping of strain A7 was performed by EIA with confirmation by genotyping, as described by Noel et al. (1995). Briefly, a portion of ORF2 of the A7 genome was amplified by reverse transcriptase (RT)-PCR using primers Mon244 and Mon245. The nucleotide sequence of the 413 bp amplicon was determined, and a 348 bp region was compared with the seven reference strains by using the Pileup program of the Genetics Computer Group sequence analysis package (Genetics Computer Group, 1994).

TABLE I. Summary of analysis of specimens of patients affected in outbreak of gastroenteritis

	Serology Assays						Stool Assays		
	Protein Dot Blot			SPIEM			PCR		ELISA
ID	Acute	Conv	Conversion	Acute	Conv	Conversion	HAstV	EV	RV
1	−	+	+	−	+	++++	+	−	−
2							−	−	−
3							−	−	−
4	−	+	+	−	+	++++	−	−	−
5							−	−	−
6							−	−	−
7	−	+	+	−	+	++++	+	−	−
8							−	−	−
9							−	−	−
10							−	−	−
11	−	−	−	−	−	−	−	+	+
12							+	+	−
13							−	−	−
14	+	+	−	−	+	+	−	−	−
15	+/−	−	−	−	+	++	−	−	−
16	−	+	+	−	+	++++	+	−	−
17	−	−	−	−	+	+	na	na	na

Acute- and convalescent-phase (conv) sera were tested for antibodies to astrovirus strain A7 by protein dot blot assay and solid phase immune electron microscopy, as described. The following code was used: 4+, strong seroconversion; 2+, low seroconversion; 1+, very low seroconversion; −, no seroconversion. Viruses were detected in stool by PCR for astrovirus (HAstV) and enterovirus (EV) and by ELISA for rotavirus (RV), na, no specimen available for testing.

Radioimmunoprecipitation Assay

A 25 cm^2 flask of Caco-2 cells was infected with astrovirus strain A7. After adsorption for 1 hour, the inoculum was replaced by fresh medium containing 2% fetal bovine serum (FBS) and incubated for 16 hours. The medium was removed, the cells were washed with PBS, and medium containing 10 μg/ml of trypsin plus 20 μCi of ^{35}S-methionine (DuPont, NEN Research) was added followed by incubation for another 24 hours at 37°C. The cell lysate was harvested by three cycles of freezing and thawing and was clarified at 1,000 rpm for 15 minutes at room temperature. Four milliliters of the supernatant were centrifuged on a cushion of 30% sucrose in TNE for 1 hour at 50,000 rpm and 5°C. The pellet was resuspended in 300 μl of PBS, pH 7.2, and was used for radioimmunoprecipitation assay (RIPA). Fifty microliters of the purified virus were diluted six-fold in PBS, pH 7.2, and 1 μl of serum was added. Following incubation overnight at 4°C, the immune complexes were precipitated by fixed *S. aureus* (Harlow and Lane, 1988). The pellet was resuspended in 30 μl of lysis buffer and boiled for 5 minutes at 100°C immediately before analysis in polyacrylamide gradient gels. The electrophoresis was performed as described by Laemmli (1970). For continuous gels and 8–20% gradient gels, acrylamide crosslinking of 3 and 2.7% were used, respectively. For RIPA, the gel was treated for fluorography, dried, and exposed to Kodak Xomat film for 3 days at −80°C.

RESULTS

Detection of Viruses in Feces

All of the stool samples were negative for enteropathogenic bacteria by routine culture. Rotavirus was detected in one stool (patient 11) by EM and was confirmed as group A by EIA (Table I). Particles with typical astrovirus morphology were detected by EM in two stools (patients 7 and 12). The stool of patient 7 contained more visible particles, so it was selected for solid-phase IEM (SPIEM) assays and further characterization.

RT-PCR was performed on RNA purified from the stool samples using oligo(dT) as the first-strand primer. This approach allowed the same cDNA to be used for both enterovirus and astrovirus PCR. Two stools were positive for enterovirus, and four were positive for astrovirus (Table I). Both of the enterovirus-positive stools were also positive for a gastroenteritis virus (either rotavirus or astrovirus).

Serologic Studies

The eight pairs of acute- and convalescent-phase sera were tested for reactivity to the astrovirus in the stool of patient 7 (A7 virus) by using SPIEM. Serum pairs from four patients, including the homologous pair, showed a strong seroconversion to the A7 virus, three showed a weaker response, and the pair from one patient showed no reactivity (Table I). Seven of the serum pairs, excluding the homologous pair, were also tested for reactivity to the cell culture-derived strain A7 by using a dot blot format (Fig. 1). The same three heterologous serum pairs with a strong seroresponse by SPIEM also exhibited a seroresponse by dot blot assay (Table I). The homologous serum pair was not tested by dot blot but was analyzed by using RIPA (see below).

In further experiments, the enterovirus that was detected simultaneously with astrovirus in the stool of patient 12 was culture adapted in Caco-2 cells. A stock

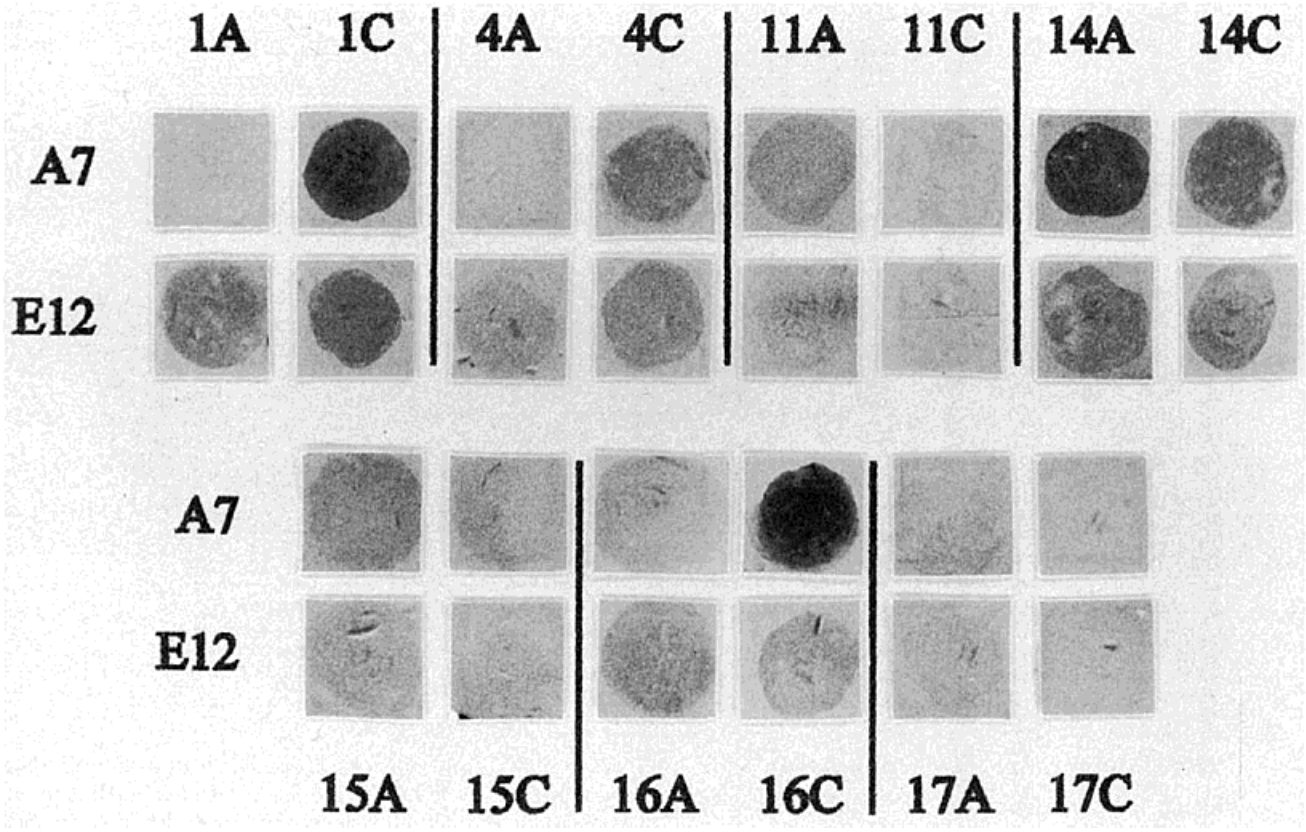

Fig. 1. Analysis by dot blot of acute-phase and convalescent-phase sera. Sera were tested by using 100 ng of astrovirus A7 and enterovirus E12 purified by isopycnic centrifugation in cesium chloride gradient. The origin of the sera is indicated under each dot blot. Human sera were diluted 1:500 and incubated overnight at room temperature (RT). Human immunoglobulins A, G, and M were detected by using peroxidase-labeled specific antihuman goat serum (Sanofi Pasteur) at 1:500 dilution. Acute-phase (A) and convalescent-phase (C) sera with their origins are indicated on each dot blot.

of this enterovirus was prepared and was used in the dot blot format as described above for astrovirus. Although reactivity to the enterovirus was detected in all acute-phase sera, no seroconversion was detected in any of the eight serum pairs tested.

Growth and Characterization of Astrovirus A7

Seven successive passages of strain A7 were performed on Caco-2 cells (Willcocks et al., 1990) with virus growth monitored by EM. The presence of trypsin in the medium was required for propagation of the virus, because no particles were observed by EM when the cultures contained 2% FBS. The culture- adapted virus was aggregated by convalescent-phase, but not by acute-phase, serum from patient 7 (data not shown). A viral stock was prepared and was purified by differential and isopycnic ultracentrifugation. A peak of astrovirus with a density of 1.36 grams/ml in CsCl was detected by EM. This purified virus was used for dot blot assay and electrophoresis in continuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

The culture-adapted strain A7 was positive in the astrovirus antigen- EIA and was determined to be serotype 3 using the serotyping-EIA as described by Noel et al. (1995). This classification was confirmed by determining the nucleotide sequence of a 348 bp region from ORF2. The percent nucleotide difference between strain A7 and the reference serotypes was HAstV-1, 23.0%; HAstV-2, 19.3%; HAstV-3, 2.9%; HAstV-4, 22.7%; HAstV-5, 21.6%; HAstV-6, 23.0%; and HAstV-8, 23.0%.

The protein composition of the purified strain A7 was analyzed by electrophoresis in SDS-PAGE. Two bands with calculated M_r of 33 and 26 kDa were detected by staining with coomassie blue (Fig. 2). The viral origin of these bands was confirmed by RIPA using rabbit antitype 3 reference serum, and acute- and convalescent-phase serum of patient 7. Both viral proteins were immunoprecipitated by the rabbit polyclonal serum and the convalescent-phase human serum (Fig. 2). A clear seroconversion was observed by blot hybridization analysis with the homologous human sera: No reactivity was detected using the acute-phase serum, and the 33 kDa protein was detected using the convalescent-phase serum.

DISCUSSION

Astroviruses were detected originally by EM examination of stools from children with diarrhea (Madeley and Cosgrove, 1975b). Sensitive assays using EIA

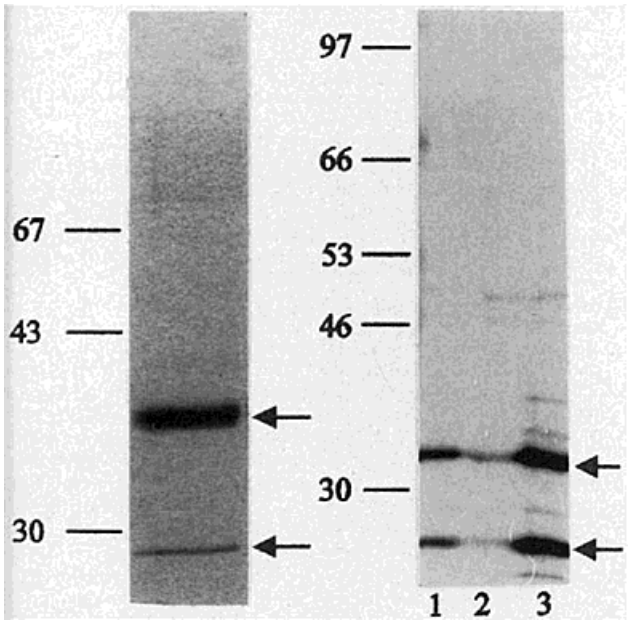

Fig. 2. **Left:** Electrophoretic pattern of capsid proteins from astrovirus A7. Astrovirus strain A7 was purified by isopycnic centrifugation in cesium chloride and the viral proteins were resolved on an 12% polyacrylamide gel and were visualized with coomassie blue staining. The molecular mass (kDa) of the marker proteins that were run in **lane M** is indicated. **Right:** Immunoprecipitation of strain A7 proteins. ^{35}S-methionine-labeled proteins from infected Caco-2 cell lysates were incubated with rabbit polyclonal antitype 3 serum (**lane 1**) or with acute-phase (**lane 2**) or convalescent-phase (**lane 3**) serum from patient 7. The immunoprecipitated proteins were analyzed on an 8–20% polyacrylamide gradient gel and were detected by fluorography. The molecular mass (kDa) of the ^{14}C-labeled marker proteins that were run in lane M is indicated.

(Herrmann et al., 1990; Moe et al., 1991; Noel et al., 1995) and PCR (Jonassen et al., 1995; Mitchell et al., 1993; Noel et al., 1995) have been developed subsequently for detecting astrovirus in stool samples. These assays, however, are not used widely, and, in many clinical settings, EM remains the only available method for the detection of astroviruses in stool sample (Monroe et al., 1991a; Lew et al., 1990).

Most studies using the newer assays have focused on the role of astrovirus as an agent of gastroenteritis in young children. One aim of the current work was to document that astrovirus was the causative agent of an outbreak of acute gastroenteritis in healthy young adults. This outbreak occurred in a military school where the students were between 18 and 20 years old. Astrovirus was detected in 2 of 16 stool samples from ill recruits by using EM and in 4 of 16 samples by using RT- PCR. A specific seroresponse to the virus in one patient's stool (A7) was demonstrated clearly in 4 of 8 serum pairs by using SPIEM and dot blot assays. Three of the four patients who had astrovirus detected in the stool sample also showed a seroconversion to A7 virus. These results confirm that astrovirus infection was associated with gastroenteritis in this outbreak in young adults.

One additional finding of the investigation of this outbreak was that multiple potential enteric pathogens were detected during the initial screening of stool samples. Rotavirus was detected in one stool by both EM and EIA, and two stools contained enterovirus that was detectable by using PCR. Although they are not commonly associated with gastroenteritis, enteroviruses may be indicators of fecal-oral contamination. Several patients in this study had antibody reactive in a dot blot assay with enterovirus E12, but none exhibited a seroconversion following the outbreak. Thus, enteroviruses were not a reliable indicator of exposure, and EV PCR may not be generally useful in analyzing specimens from outbreaks of gastroenteritis.

The second part of this study characterized the astrovirus associated with the outbreak. Serotyping and genotyping assays determined that this virus, A7, was a serotype 3 astrovirus, a type that has been detected infrequently in three surveys (Lee and Kurtz, 1994; Noel and Cubitt, 1994; Noel et al., 1995). The analysis of the protein composition by PAGE demonstrated that strain A7 is composed by two proteins, 32 and 26 kDa, the larger being the major component. Both proteins were reactive with rabbit and convalescent-phase human antisera in a RIPA, but only the larger protein was recognized by human convalescent-phase serum in an immunoblot assay. Thus, it appears that the larger protein contains the predominant linear epitopes recognized by human sera.

The capsid protein composition of strain A7 differs from that reported previously for the reference strains HAstV-1 (Willcocks et al., 1990), HAstV-2 (Monroe et al., 1991b; Sanchez-Fauquier et al., 1994), and HAstV-5 (Midthun et al., 1993). Three structural proteins were detected in the two former strains, whereas only a single capsid protein was reported for serotype 5. The different pattern for strain A7 could be explained by several hypothesis, including 1) the major band was actually composed by two proteins that comigrated, even using a gradient gel system; 2) the loss of a third protein during virus maturation or purification; or 3) the processing pathway of the viral capsid precursor differs among serotypes. These hypotheses could also explain the single 30 kDa structural protein reported for serotype 5. A recent analysis of the predicted sequences of astrovirus capsid precursors indicated that they differ in size and contain regions of high sequence divergence between serotypes (Willcocks et al., 1995). It remains to be determined whether there is a consistent pattern of protein composition within serotypes. A further comparison of capsid composition of wild strains and reference strains should provide the answer.

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REFERENCES

Genetics Computer Group (1994): Program manual for the Wisconsin package, version 8. Madison: Genetics Computer Group.

Grohmann GS, Glass RI, Pereira HG, Monroe SS, Hightower AW, Weber R, Bryan RT (1993): Enteric viruses and diarrhea in HIV-infected patients. New England Journal of Medicine 329:14–20.

Harlow E, Lane D (1988): "Antibodies: A Laboratory Manual." New York: Cold Spring Harbor Laboratory.

Herring AJ, Gray EW, Snodgrass DR (1981): Purification and characterization of ovine astrovirus. Journal of General Virology 53:47–55.

Herrmann JE, Blacklow NR, Perron- Henry DM, Clements E, Taylor DN, Echeverria P (1988): Incidence of enteric adenoviruses among children in Thailand and the significance of these viruses in gastroenteritis. Journal of Clinical Microbiology 26:1783–1786.

Herrmann JE, Nowak NA, Perron-Henry DM, Hudson RW, Cubitt WD, Blacklow NR (1990): Diagnosis of astrovirus gastroenteritis by antigen detection with monoclonal antibodies. Journal of Infectious Diseases 161:226–229.

Jiang B, Monroe SS, Koonin EV, Stine SE, Glass RI (1993): RNA sequence of astrovirus: Distinctive genomic organization and a putative retrovirus-like ribosomal frameshifting signal that directs the viral replicase synthesis. Proceedings of the National Academy of Sciences, USA 90:10539–10543.

Jonassen TO, Monceyron C, Lee TW, Kurtz JB, Grinde B (1995): Detection of all serotypes of human astrovirus by the polymerase chain reaction. Journal of Virological Methods 52:327–334.

Kurtz JB, Lee TW (1984): Human astrovirus serotypes (letter). Lancet ii:1405.

Kurtz JB, Lee TW (1987): Astroviruses: Human and animal. In Bock G, Whelan J (eds): "Ciba Foundation Symposium 128. Novel Diarrhoea Viruses." New York: John Wiley and Sons, pp 92–107.

Kurtz JB, Lee TW, Craig JW, Reed SE (1979): Astrovirus infection in volunteers. Journal of Medical Virology 3:221–230.

Laemmli UK (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685.

Lee TW, Kurtz JB (1981): Serial propagation of astrovirus in tissue culture with the aid of trypsin. Journal of General Virology 57:421–424.

Lee TW, Kurtz JB (1994): Prevalence of human astrovirus serotypes in the Oxford region 1976- 1992, with evidence for two new serotypes. Epidemiology and Infection 112:187–193.

Lew JF, Glass RI, Petric M, LeBaron CW, Hammond GW, Miller SE, Robinson C, Boutilier J, Riepenhoff-Talty M, Payne CM, Franklin R, Oshiro LS, Jaqua M-J (1990): Six year retrospective surveillance of gastroenteritis viruses identified at ten electron microscopy centers in the United States and Canada. Pediatric Infectious Disease Journal 9:709–714.

Lewis DC, Lightfoot NF, Cubitt WD, Wilson SA (1989): Outbreaks of astrovirus type 1 and rotavirus gastroenteritis in a geriatric inpatient population. Journal of Hospital Infections 14:9–14.

Lewis TL, Matsui SM (1995): An astrovirus frameshift signal induces ribosomal frameshifting in vitro. Archives of Virology 140: 1127–1135.

Lewis TL, Greenberg HB, Herrmann JE, Smith LS, Matsui SM (1994): Analysis of astrovirus serotype 1 RNA, identification of the viral RNA-dependent RNA polymerase motif, and expression of a viral structural protein. Journal of Virology 68:77–83.

Madeley CR, Cosgrove BP (1975a): Twenty-eight nanometer nm particles in faeces in infantile gastroenteritis. Lancet ii:451–452.

Madeley CR, Cosgrove BP (1975b): Viruses in infantile gastroenteritis [letter]. Lancet ii:124.

Marczinke B, Bloys AJ, Brown TDK, Willcocks MM, Carter MJ, Brierley I (1994): The human astrovirus RNA-dependent RNA polymerase coding region is expressed by ribosomal frameshifting. Journal of Virology 68:5588–5595.

Midthun K, Greenberg HB, Kurtz JB, Gary GW, Lin F-Y, Kapikian AZ (1993): Characterization and seroepidemiology of a type 5 astrovirus associated with an outbreak of gastroenteritis in Marin County, California. Journal of Clinical Microbiology 31:955–962.

Mitchell DK, Van R, Morrow AL, Monroe SS, Glass RI, Pickering LK (1993): Outbreaks of astrovirus gastroenteritis in day care centers. Journal of Pediatrics 123:725–732.

Mitchell DK, Monroe SS, Jiang X, Matson DO, Glass RI, Pickering LK (1995): Virologic features of an astrovirus diarrhea outbreak in a day care center revealed by reverse transcription-polymerase chain reaction. Journal of Infectious Diseases 172:1437–1444.

Moe CL, Allen JR, Monroe SS, Gary HE, Jr, Humphrey CD, Herrmann JE, Blacklow NR, Carcamo C, Koch M, Kim K-H, Glass RI (1991): Detection of astrovirus in pediatric stool samples by immunoassay and RNA probe. Journal of Clinical Microbiology 29:2390–2395.

Monroe SS, Glass RI, Noah N, Flewett TH, Caul EO, Ashton CI, Curry A, Field AM, Madeley R, Pead PJ (1991a): Electronmicroscopic reporting of gastrointestinal viruses in the United Kingdom, 1985–1987. Journal of Medical Virology 33:193–198.

Monroe SS, Stine SE, Gorelkin L, Herrmann JE, Blacklow NR, Glass RI (1991b): Temporal synthesis of proteins and RNAs during human astrovirus infection of cultured cells. Journal of Virology 65:641–648.

Monroe SS, Jiang B, Stine SE, Koopmans M, Glass RI (1993): Subgenomic RNA sequence of human astrovirus supports classification of Astroviridae as a new family of RNA viruses. Journal of Virology 67:3611–3614.

Noel J, Cubitt D (1994): Identification of astrovirus serotypes from children treated at the Hospitals for Sick Children, London 1981–1993. Epidemiology and Infection 113:153–149.

Noel JS, Lee TW, Kurtz JB, Glass RI, Monroe SS (1995): Typing of human astroviruses from clinical isolates by enzyme immunoassay and nucleotide sequencing. Journal of Clinical Microbiology 33:797–801.

Oishi I, Yamazaki K, Kimoto T, Minekawa Y, Utagawa E, Yamazaki S, Inouye S, Grohmann GS, Monroe SS, Stine S, Carcamo C, Ando T, Glass RI (1994): A large outbreak of acute gastroenteritis associated with astrovirus among students and teachers in Osaka, Japan. Journal of Infectious Diseases 170:439–443.

Risco C, Carrascosa JL, Pedregosa AM, Humphrey CD, Sanchez-Fauquier A (1995): Ultrastructure of human astrovirus serotype 2. Journal of General Virology 76:2075–2080.

Sanchez- Fauquier A, Carrascosa AL, Carrascosa JL, Otero A, Glass RI, Lopez JA, San Martin C, Melero JA (1994): Characterization of a human astrovirus serotype 2 structural protein (VP26) that contains an epitope involved in virus neutralization. Virology 201:312–320.

Shimizu M, Shirai J, Narita M, Yamane T (1990): Cytopathic astrovirus isolated from porcine acute gastroenteritis in an established cell line derived from porcine embryonic kidney. Journal of Clinical Microbiology 28:201–206.

Willcocks MM, Carter MJ, Laidler FR, Madeley CR (1990): Growth and characterisation of human faecal astrovirus in a continuous cell line. Archives of Virology 113:73–81.

Willcocks MM, Brown TDK, Madeley CR, Carter MJ (1994): The complete sequence of a human astrovirus. Journal of General Virology 75:1785–1788.

Willcocks MM, Kurtz JB, Lee TW, Carter MJ (1995): Prevalence of human astrovirus serotype 4: capsid protein sequence and comparison with other strains. Epidemiology and Infection 114:385–391.

Zoll GJ, Melchers WJ, Kopecka H, Jambroes G, van der Poel HJ, Galama JM (1992): General primer-mediated polymerase chain reaction for detection of enteroviruses: application for diagnostic routine and persistent infections. Journal of Clinical Microbiology 30:160–165.